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STUDIES ON PLASMOPARA VITICOLA.

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Historical.

Early writers frequently mention the "mildews" and "blights" which "sickeneth" the vines but these troubles were attributed to some visitation of Providence or to atmospheric disturbances. As late as 1870 the disease was not supposed to be caused by any organism, although a conjunct fungus was always found. Saunders (1862) was of the opinion that the vine was previously injured and partially decayed before the fungus was able to gain foothold.

Lippincott (1866) in a summary of the various theories of the origin of the disease held at that time, states that they were essentially as follows: that it is spontaneously developed, that the spores are inhaled through the stomates and distributed throughout the plant and that during moist weather the plants become gorged with water resulting in an incipient stage of decomposition so weakening the plants that they are unable to prevent the formation of the mildew. Lippincott, himself, offers the explanation that ozone in the air prevents the formation of the mildew and that during moist weather this prophylactic gas is not present, hence the abundance of the disease during such periods.

Farlow (1876) described the disease and correctly attributed it to the fungus, Peronospora viticola De Bary.

Name and Classification.

The fungus was first collected in America by Schweinitz, in 1834, but was erroneously referred by him to Botrytis cana Link. It was studied by Berkeley and Curtis (1848) who described it as a new species, Botrytis viticola. De Bary (1863) later studied the fungus carefully, described and figured the sexual as well as the asexual stage and referred it to the genus Peronospora, naming it Peronospora viticola.

Schroeter (1886) subdivided the genus Peronospora into Peronospora and Plasmopara. The differences may be briefly stated. The conidiophores of Plasmopara are monopodially branched, never clearly dichotomously as in Peronospora. The ultimate branches or sterigmata of Plasmopara are dichotomous or trichotomous and after the dissemination of the conidia their ends are flat or concave. In Peronospora the sterigmata are always dichotomous and the ends are acutely or obtusely pointed but never flat. Finally the germination of the conidia of Plasmopara is typically by means of zoospores or by the entire contents of the spore slipping out and then producing a germ-tube. Rarely a germ-tube may be produced directly. The conidia of Peronospora always germinate by means of a germ-tube.

Berlese and de Toni (1888) redescribed the fungus as Plasmopara viticola.

Characterization.

The mycelium is cœnocytic and pleuroblastic. Istvanffi (1913) states that it may sometimes be intracellular and figures it so. The writer has never observed the mycelium in a position which might be interpreted as intracellular. Since all observations were made from stained sections which were not more than 10μ thick all possibility of mistaking hyphae passing

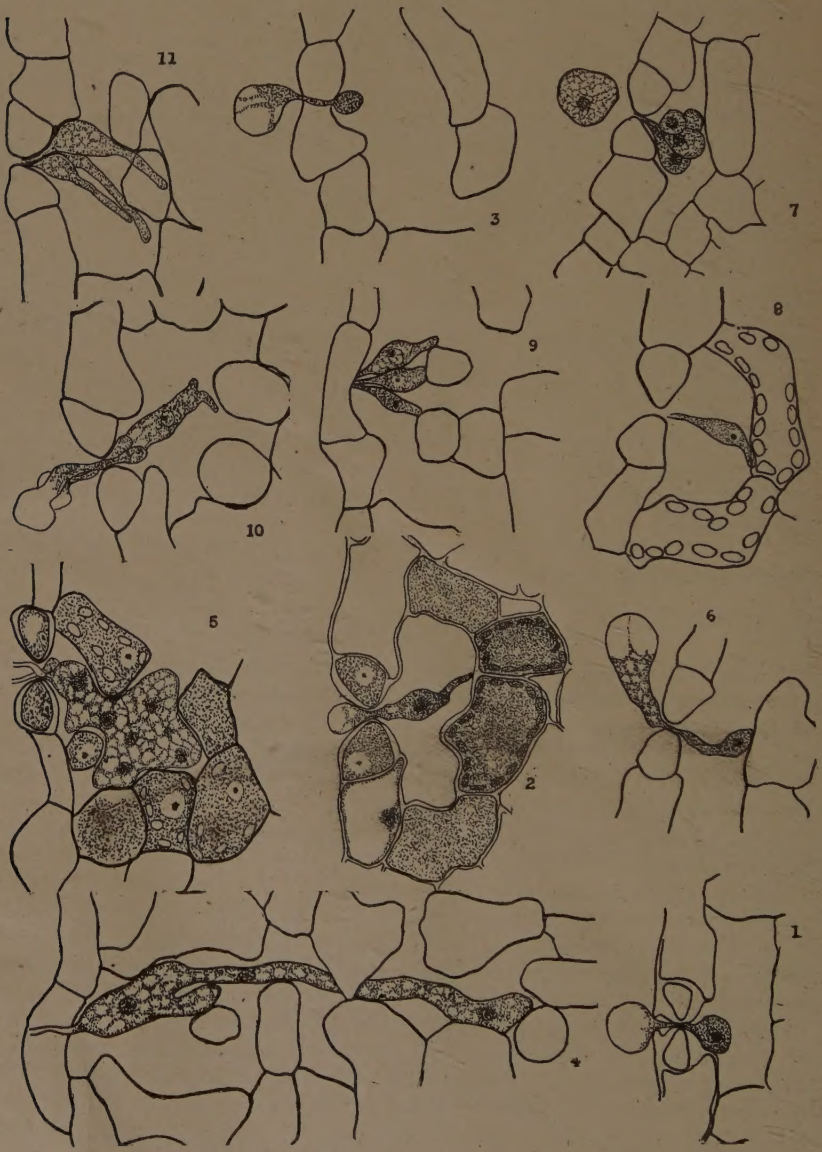

Plate I. Infection of grape leaf by Plasmopara viticola.

above or below the cell, as being intracellular, is obviated. In the writer's opinion the mycelium is always intercellular.

The diameter of the hyphae varies considerably with the conditions of temperature and moisture and with the character of the tissue in which it is located. Where the germ-tube and the conidiophore pass through the stomate it is about 1μ in diameter (Pl. I, fig. 1). In certain cases the diameter of the mycelium may be as much as 40 to 60μ (Pl. I, fig. 5). In the canes where the tissues are firm and the cell walls relatively thick, the hyphae are smaller and of more uniform diameter. In the leaves the hyphae tend to fill the large, irregular, intercellular spaces and hence are of greater diameter. In general it may be said that in young tissue the mycelium is of greater diameter and more abundant than in the more mature parts. Indeed, in the very old, diseased areas, particularly after the period of oospore formation, the mycelium seems to disappear almost entirely.

The younger parts of the mycelium are filled with a dense, finely granular protoplasm which, with age, becomes coarser and profusely vacuolated. When carefully stained the protoplasm exhibits a netted structure seemingly made up of numerous fine strands. At irregular intervals upon these strands occur deeper staining granules whose exact nature is not evident.

The mycelial wall is very thin and according to Mangin (1890), is composed of a mixture of cellulose and callose. The action of various stains may serve as an indication of its nature. When untreated mycelium is stained with chloriodide of zinc there appears after a short time a brown color. If previously boiled in potassium hydroxide the mycelium becomes a deep reddish purple with this stain. This is in accordance with Mangin's statement to the effect that boiling potassium hydroxide will dissolve the masking callose leaving the pure cellulose.

The nuclei are commonly globose but in certain places they may be oval or at times almost linear. They are especially abundant in the younger portions of the mycelium. Istvanffi states that they divide karyokinetically.

The haustoria are produced very profusely and occur in all parts of the plant in which the mycelium is to be found. In the leaves of the resistant varieties the haustoria are similar in every respect to those found in the more susceptible vines except that they are usually smaller and are never so abundant.

The method of haustorial formation is similar to that described by Grant Smith (1900) for the Erysiphae. At first a very small tube is produced laterally from the mycelium into the host cell-wall which becomes greatly thickened at this point. The exact cause of this thickening is not apparent. It would seem that, if it is caused by some enzyme producing gelatinization of the wall, as is suggested by Cuboni (1889), the adjacent portions of the wall would also swell but this is not the case. The other possible view is that it is an indication of an attempt of the host to exclude the haustorium hence is the result of additional depositions at this point by the protoplasm of the irritated cell. Be this as it may, the haustorium continues to grow inward still enclosed in its sheath of host wall. After reaching a variable length the end of the tube gradually swells into a globose sac accompanied to a certain point by the swelling of its sheath (Pl. II, fig. 1). After attaining about one-half of its mature size the terminal portion bursts or dis-

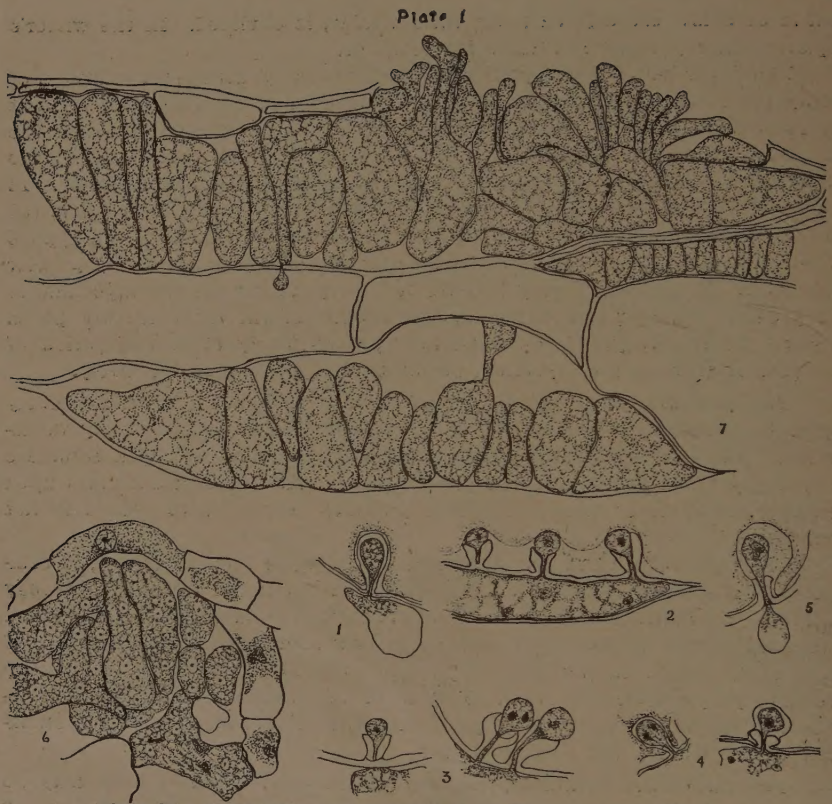

Plate II. Haustoria and mycelium of Plasmopara viticola.

solves the sheath. Thus in many of the mature haustoria there may be seen about the base a goblet-shaped collar pierced by the greatly attenuated stem of the haustorium (Pl. II, figs. 2 and 3). After penetrating the cell wall, the terminal portion continues its growth for a certain period, never, however, completely filling the cell as is sometimes the case in certain of the Erysiphaceae. It is this terminal, globose portion which constitutes the absorbing area of the haustorium.

In the meantime the plasma membrane of the cell is gradually pressed back by the advancing haustorium and often may be very closely appressed to the latter but never penetrated by it (Pl. II, figs. 2 to 5).

The haustorium is thin walled. The presence of the host cell wall about the partially swollen tip or the space between the plasma membrane and the haustorium may give the impression that the wall is thick as has been stated by Istvanffi (1913) and others. The former states that the wall is sometimes thin and sometimes very thick. It is evident that in the first instance he has observed the condition in which the plasma membrane is closely appressed to the haustorium and in the second, the encompassing sheath of host cell-wall.

Within the haustorium is a small bit of protoplasm in which is imbedded a nucleus. In certain cases the writer has observed two nuclei (Pl. II, figs. 3 to 5). Istvanffi (1913) has reported as many as four. In his estimation these are produced by the division of a single nucleus which entered the haustorium during the early stages of its development.

The size of the haustoria is exceedingly variable. According to Istvanffi they are 4 to 10μ (rarely 15 to 20μ) at their largest diameter by 4 to 12μ (rarely 20 to 25μ) long. These measurements correspond very closely with those made by the writer. The stems may attain nearly one-half of the total length of the haustorium, varying from 1.5 to 5.5μ in length and from .4 to 1.0μ in diameter.

The method of differentiating the cell wall from the haustorium was by the combination of certain stains. After some experimentation, it was found that ruthenium red and methyl green gave the clearest coloration. In this way the cell walls were tinted a deep red and the mycelium green. The safranin—gentian violet—orange stain also gives very good differentiation showing the relation to the plasma membrane but since it leaves the cell-wall practically unstained it is unsuitable for that aspect of the problem. A study of sections stained by both methods gives an excellent idea of the relation of parts.

The formation of conidiophores commences after a sufficient period of mycelial development, the time varying with the organ attacked, the temperature and the humidity. The mycelium begins to mass beneath the stomata forming what has been termed the cushion (Pl. II, fig. 6). From these hyphae arise many other smaller ones which push upward through the stomata. In order to traverse the small opening they must become greatly attenuated but immediately after their egress they abruptly swell to the normal size of the conidiophore. As many as twenty such branches may arise through a single stomate but more commonly there are from four to six.

In certain cases the fruiting mycelium may burst directly through the epidermis. This has been observed frequently on flower peduncles which were heavily infected (Pl. II, fig. 7). In such cases it appears that the epidermal cells are killed, then crushed by the pressure of the mycelium beneath and finally disrupted. There is no evidence that it is a process of solution. On the young fruit the mycelium takes advantage of the lenticle-like structures for its egress.

The conidiophores are produced most readily in the absence of light, in relatively humid conditions and at a temperature of 18° to 20° C. The other conditions being favorable the temperature is of rather minor importance. The method ordinarily employed to obtain an abundance of conidia for germination studies is to enclose infected leaves, on which there is no evidence of conidiophore formation, in a moist chamber during the night (about 12 to 20 hours). A smaller percentage of conidia frequently have been obtained during the day by a similar treatment. The light was in such cases very subdued. This is in accordance with the statements of Istvanffi (1913), Cuboni (1889) and others to the effect that conidiophores are produced only during the night or in subdued light.

The conidiophores are from 300 to 500μ in height and 7 to 9μ in diameter. Istvanffi states that they average 800 to 1200μ in height and that occasionally one may attain the height of 1500μ. There are usually about six monopodial

branches which are in turn branched several times. Each branch is terminated by two to four, short sterigmata whose ends, after the dispersal of the conidia, are concaved. The sterigmata are about 5 to 12μ long and 2 to 3μ in diameter.

The walls of the conidiophores are thicker than those of the mycelium and are composed at least partially of cellulose, staining a bluish purple with zinc chloriodide. At irregular intervals in the primary axis, branches or the sterigmata, there occur septa which are very much thicker than the walls. These are claimed by Mangin (1890) not to be true cross-walls but merely plugs of callose. When stained with zinc chloriodide they are not tinted but, as in the case of the mycelium, do so after the conidiophore has been boiled in potassium hydroxide.

To determine whether the conidiophores are composed of pure cellulose they were treated with a cellulose solvent, cuprammonia (Schweizer's reagent). A preliminary observation was made of the action of this solvent upon cotton fibers (pure cellulose). The first evidence of its action is a swelling of the fibers to at least double their original diameter, followed shortly by their rapid disappearance. The walls of the conidiophores, when treated in the same manner, also swelled to about twice their normal thickness but they do not then rapidly dissolve (Pl. III, fig. 1). The basal portion exhibits a peculiar reaction, seeming to burst outward and to disintegrate except for a thin inner sheath (Pl. III, figs. 1 and 3). Sometimes the large proportion of the conidiophore assumes this aspect, but in practically every case the distal end remains apparently intact. A logical interpretation of these reactions is that the conidiophores are composed of cellulose or a cellulose-like substance but are lined with another material, possibly callose.

Before the formation of the conidia, the conidiophore is filled with protoplasm which is abundantly provided with nuclei. The conidia are formed by the swelling of the end of the sterigmata into which a portion of the protoplasm passes. After the mature size is attained a septum is laid down separating the conidium from the sterigma. According to Istvanffi (1913) a single nucleus passes into each spore at this time and its division produces the multinucleate condition obtaining in the mature conidium. He also states that the cross-wall is laid down when the conidium is from one-half to two-thirds its mature size.

The mature conidia are extremely variable in size and shape. Some are almost globose, others are long and narrow, but the majority are ovoid. They are attached to the sterigmata at their smallest end. Disarticulation occurs very easily, being produced by the breaking or solution of a small lenticular area which separates the conidium from the sterigma (Pl. III, fig. 5). The ease with which this substance is broken is shown by the fact that only a slight jarring is necessary to release the spore. It is also very readily soluble in water hence the exceeding difficulty of obtaining a mount in water of the attached spores for microscopic examination. One may observe many conidia still fastened to their sterigmata when in the dry state but if a drop of water is placed upon the mass in a moment all of the conidia will be freed.

Cornu (1882) mentions this substance and states that it is due to its extreme solubility that the conidia are freed during moist weather. He figures it, however, as a wall which is so tightly compressed between the

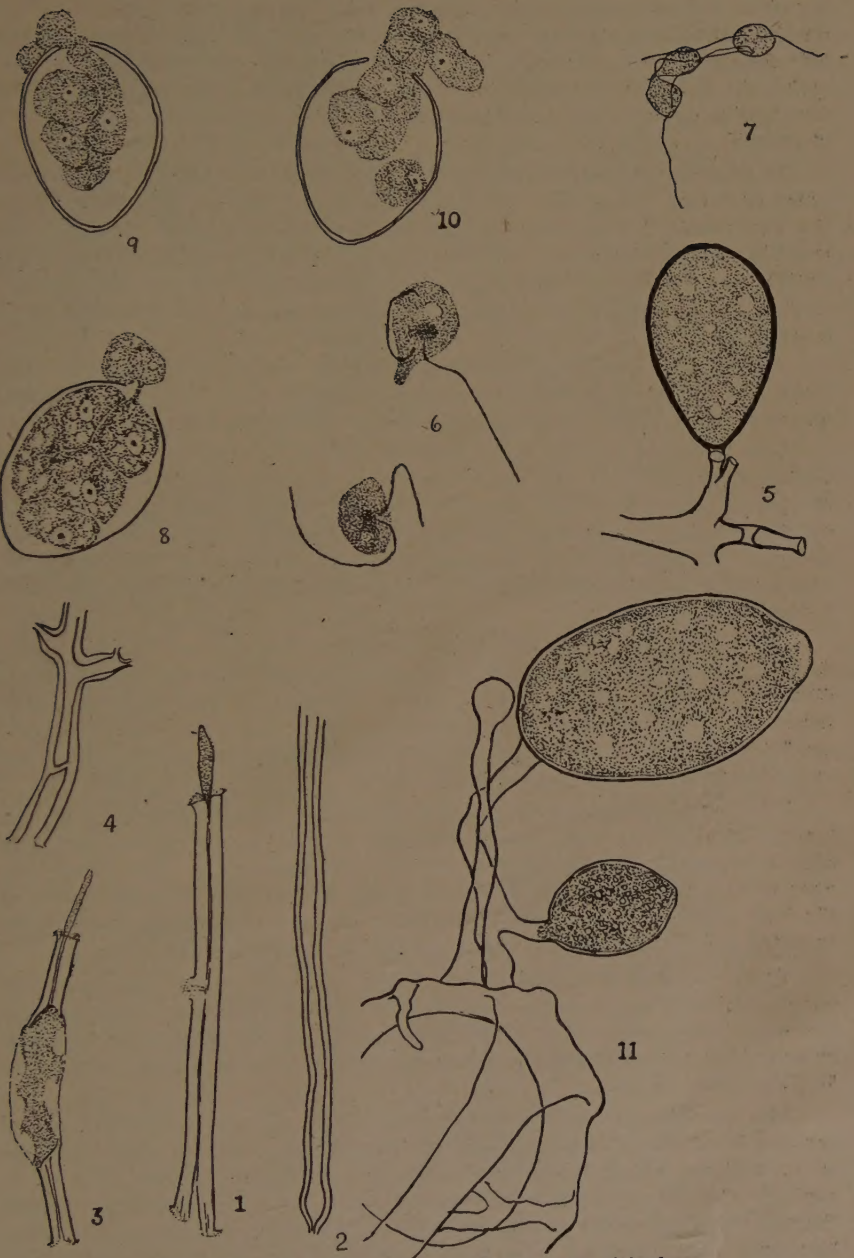

Plate III. Fructifications of Plasmopara viticola.

conidium and the sterigma that it bulges out on either side. Mangin (1891) on the other hand, having made a complete study of this subject, figures this disjunctive wall as lenticular. He claims that it is composed of callose which is, however, insoluble in water but explains this anomaly by the theory that there is some chemical change in the composition which renders it soluble.

In size the conidia average from 11 to 18μ by 15 to 31μ but in certain cases are much larger than this. Istvanffi (1913) distinguishes three types, the microconidia, which are of the size and shape usually observed, the macroconidia, having a size of 25 to 35μ by 40 to 55μ and finally the megaloconidia which are much larger than the macroconidia, more or less globose, contain 15 to 20 nuclei and which are produced without the intervention of a sterigma.

At the small end of the conidium there are usually two minute projections, the remnants of the attachment to the sterigma. At the opposite, larger end is a papilla or projection through which germination occurs.

The thickness of the wall of the conidium lies between that of the conidiophore wall and the mycelium. It is practically uniform except at the papilla where it is very thin and delicate. The composition is the same as that of the conidiophore, staining reddish-purple with zinc chloriodide except at the papilla which remains colorless. Within each conidium there are several nuclei corresponding to the zoospores which are ultimately formed in it.

On susceptible varieties, like the Delaware, Niagara and V. bicolor, the downy mass of conidiophores and conidia is usually very dense and when disturbed, a tiny white cloud of spores arises. On the more resistant forms the growth is only slightly developed even under the most favorable conditions, appearing most frequently in isolated clusters which correspond to the brown punctations on the upper surface.

The sexual spores, oospores, are formed in the intercellular spaces largely within the palisade or spongy parenchyma tissues of the leaf, and almost invariably closely adjacent to the principal veins. The writer has very seldom found them in the tissue about the margin or in the blade of the leaf. Bailion (1888), Istvanffi (1913) and Lamson-Scribner (1886) have reported finding them in the cortex of the stem and in the berries.

The development of the oospores has not been clearly followed but several different stages have been observed and in order that these may be correctly interpreted and arranged in their proper sequence, a comparison is made here with the oospore development of Plasmopora alpinum, described by Rosenberg (1903).

The oogonium is multinucleate (Pl. IV, fig. 1) and number of nuclei counted in one case being about forty. This is very similar to the oogonium of P. alpinum which Rosenberg states has forty-five. In the case of P. alpinum the antheridia are clavate and contain about four or five nuclei. Though many hundreds of stained sections have been examined, the writer has only once observed a structure which might be interpreted as an antheridium. It was much smaller than the oogonium, clavate and arose from the hyphaœ immediately beneath the former (Pl. IV, fig. 2). Both Viala (1893) and Cornu (1882) figure similar structures but Farlow (1876)

Plate IV. A few stages in the development of the oospore.

represents the antheridium as a long, slender thread partially encircling the oogonium.

Briefly stated the nuclear history of the oogonium of P. alpinum is as follows. First, there is a single mitotic division, after which all of the nuclei but one migrate to the periphery. Simultaneously there appears at the center a small dense body which seems to attract the single remaining nucleus to it. This is considered by Rosenberg to be the coenocentrum. Following this, another mitosis of the central nucleus occurs, which does not, however, occur in all of the nuclei at the periphery. One of the sister nuclei at the center then migrates to the periphery and gradually disintegrates. At this time one nucleus from the antheridium passes to the center of the oogonium and after a time fuses with the female nucleus.

In Plasmopara viticola, certain of these stages have been observed. In many instances a single, well-developed nucleus occurs at the center near a well-defined, denser, body, the coenocentrum (Pl. IV, figs. 3 and 4) while outside of the partially developed oospore wall are numerous irregular, darker-staining bodies which may be interpreted as disintegrating nuclei (Pl. IV, figs. 3 and 4). At times there may be seen two or more nuclei within the developing oospore but the fully matured oospore contains only a single nucleus without the accompanying coenocentrum (Pl. IV, fig. 6).

The writer has never observed an antheridium in connection with the more advanced stages of development of the oospore in the many hundreds of sections obtained. This fact is amenable to two interpretations, either that these oospores develop apogamously or that the antheridia very quickly disintegrate.

The protoplasm of the oospore is granular, rather yellowish brown and filled with numerous, refringent globules. At times the entire center is occupied with a large, round, hyalin area, having the appearance of a large vacuole. The oospores are from 25 to 35μ in diameter. The inner wall or endosporium of the oospore is smooth, colorless and relatively thick. This wall is formed about the oosphere immediately following fertilization. It is apparently a deposition from the protoplasm (Pl. IV, fig. 5). Surrounding the endosporium are the disintegrating remnants of nuclei lying in the periplasm. It is probable that the periplasm contracts about the endosporium thus forming the irregular, brown exosporium. The oospore lies within the thickened persistent, oogonial wall. The writer has seen many oospore-like bodies, having spiny walls but since they are as frequently on the outside of the leaf as within it is apparent that they are not spiny forms of the oospores of Plasmopara viticola.

As has been pointed out by the writer (1913) there were many unsuccessful attempts to germinate the oospores of Plasmopara viticola but, notwithstanding these failures, two theories were evolved as to the probable method of germination. On the one hand we find Millardet (1883), Frechou (1885), Richon, Viala (1893) and others who considered that zoospores were formed directly from the protoplasm of the oospore. On the other hand, Cornu (1882) and Prillieux (1883) were equally certain that the oospores produced a typical conidiophore bearing conidia.

The writer (1913) published an account of germination by means of a promycelium bearing a single conidium (Pl. III, fig. 11). Shortly afterwards,

Ravaz and Verge (1913) also described an exactly similar method fully corroborating these observations.

Oospore germination may begin as early as the latter part of February, though at this time it is not as abundant as later in the spring, March till June.

The oospores will retain their vitality for at least a year. Leaves containing oospores were collected in October, 1912, allowed to remain on the ground during the winter of 1912-13 and then, kept in the laboratory in a thoroughly dried condition until March, 1914. At this time they were moistened and after being maintained about three weeks in these conditions germination occurred in a small percentage of the oospores.

Freezing seems to be necessary, since germination has only been obtained from those oospores which have been allowed to remain on the ground until at least January or February.

The conditions essential for germination are very similar to those necessary for conidial germination. A temperature of about 23° C. and an abundant supply of moisture seems to be optimum. The leaves are allowed to rot for a week or more in a warm moist place and thereafter it requires usually about five to eight days in order to obtain germination.

The time necessary for the complete formation of the primary conidium and the emission of the zoospores is about 24 hours. The germination of two oospores was followed from the time that the conidia were 8μ and 11μ respectively in diameter at 12:35. The promycelium was still completely filled with protoplasm. At 4:45 both conidia were full size, being 27.3μ and 39μ respectively in diameter and the septum separating them from the promycelium was formed. The next morning they were in a similar condition but at 12 m. germination, by means of zoospores, occurred.

Numerous motile bodies have been observed in cultures which apparently contained no germinating oospores and it was at first thought that possibly germination might occur by zoospores directly but when these motile bodies were fixed in osmic acid and stained with methylene blue it could be seen readily that they possessed but one flagellum. Furthermore, their globose shape and size (10 to 13μ) preclude the possibility of their being zoospores of Plasmopara viticola. Melhus (1914) has observed a parasite of oospores which produces similar zoospores, hence it may be that we are also dealing here with such a parasite.

With these points in mind it appears certain that early spring infections arise from the oospores. Though the process has not been followed, certain observations have indicated that this is the case.

The leaves lying on the ground about the vines during the winter, are in an advanced stage of decomposition by spring, thus only a short period of warm, rainy weather is necessary to complete this process. The driving rains, also occurring at this time, aid in breaking up the thoroughly rotted leaves, thus more or less completely exposing the oospores and facilitating conidial formation and dissemination.

The conidia may germinate in the soil water or may themselves be spattered to the lower leaves of the vine during heavy rains. Arbois de Jubainville (1883) has stated that he has seen mildewed spots on the leaves opposite bits of mud. The writer has also observed the mildew in the spring on the mud-bespattered leaves. This would indicate that some spores, either

zoospores or conidia were carried there through the agency of the rain. It is, moreover, well known that the first spots to appear in the early spring or summer are on the lower leaves.

Millardet thought that the seedlings growing through the layers of oospore-filled leaves are infected by the zoospores produced in the oospores and the conidia resulting from these primary infections serve as sources of infections upon the surrounding vines. This hypothesis, with the exception of the oospore germination by zoospores, may be tenable in France, but in this country where seedlings never, or at least very seldom, appear in the vineyards, we must search for some other explanation.

Noack (1899) states that in Brazil the oospores are unnecessary since the leaves are green the year round and the summer stage is constantly present.

At one time the writer thought that possibly the mycelium of the fungus could hibernate in the roots or canes of the diseased vine and, producing conidia in the spring, serve as a source of infection. During the autumn of 1912 roots and canes of certain vines which were known to be badly infected each year were planted in the greenhouse, care being taken not to bring in soil which might contain oospores.

These vines grew until the following July without any evidence of the mildew, though they were well watered and an attempt made to induce a succulent growth which would probably favor any mycelial development.

Istvanffi (1913) found the mycelium and conidia within the bud scales in autumn but was not able to prove that a diseased cane or leaves develop from these buds, as Cuboni (1889) thought possible.

Frechou (1885) states that the mycelium may hibernate in the leaves which do not become moist enough to rot. After five or six months this mycelium may produce conidiophores bearing conidia. He points out that it is extremely rare that there will not be enough moisture during the autumn, winter and spring to rot the leaves, hence this method need hardly be seriously considered.

During the winter of 1914 the writer had an opportunity to test the possibility of such a method of hibernation. Leaves of a wild vine which were known to have been badly infected, were found in a fine state of preservation, showing no evidence of decomposition and in every way ideal for hibernation of the mycelium as described by Frechou. They were placed in a warm, moist situation, in the hope that conidiophores would be produced but without success, indicating that the mycelium does not hibernate in this manner.

It is probable that the principal, if not the only, method of hibernation is by means of the oospores.

After the formation of the first crop of conidia from the primary infections the spread of the mildew becomes much more rapid. The conidia are blown to other vines where they readily germinate if proper conditions exist. The principal factors influencing the germination of the conidia, aside from the presence of moisture are, the temperature, and the proper age of the conidium.

Viala (1893) gives three types of germination of the conidia, namely, by zoospores, by the emission of the entire contents of the conidium and by a germ-tube directly. Istvanffi (1913) also figures the same types and states

that germination by means of the germ-tube occurs only in moist air. The writer has never observed this type. In the great majority of cases zoospores are formed. Very rarely the entire contents of the conidium emerges, forms a wall about itself, and eventually produces a tube.

In one case a number of conidia were observed which produced several amoeba-like bodies, emerging in each case from the apical end of the conidium. After a short period, during which they moved about in the typical amoeba-like manner, they came to rest and became globose as do the zoospores.

According to Viala (1893), the optimum temperature for germination is from 28° to 30° C. At this temperature the zoospores are produced in about one-half hour. At temperatures from 10° to 17° C., germination only occurs after two or three days and a temperature of 2° to 5° C., prevented germination entirely. Istvanffi (1913), on the other hand, states that the optimum temperature for germination is from 20° to 22° C., while at 28° to 30° C. germination practically ceases, being feeble at the end of six to ten hours. He found that after two or three hours there is profuse germination at a temperature of 14° to 15° becoming slight at 8° C. Melhus (1911) states that a temperature of 10° C. is most favorable to conidial germination.

During the summer of 1912 numerous attempts were made to germinate the conidia at room temperatures, 70° to 80° F., and often as high as 90° F. In some cases there was absolutely no germination after two or three days. All variety of condition were given except to change the temperature because at this time facilities were lacking for that type of experiment.

In 1913 an ice-box was used in which a temperature of 50° F. easily could be maintained. At this temperature fully 95 per cent germination was always obtained in two to six hours, averaging about three hours. At 70° F. only 40 to 50 per cent of the conidia germinated, in about one-third of the time, however, while at 80° to 90° F. there was no germination. When the slides holding the spore-laden drops were placed directly upon the ice, thus obtaining a temperature of 35° to 41° F., a slight germination by means of zoospores occurred in about twenty-four hours. Another point which was observed concerning the spores maintained at low temperature is that the zoospores continue active for a much longer time than at the higher temperatures. In the latter case the writer (1913) has found that it is common for them to rest in about thirty minutes and to have completely germinated in three to twelve hours. In the former, the zoospores are still active from twenty-four to thirty hours after germination. In the opinion of the writer the optimum temperature for germination lies between 50° and 60° F.

Speaking of the effect of light upon the conidia Cuboni (1889:33) says: "the conidia which have been exposed to an intense light for some time, according to my observations, are sterilized and unable to germinate." Istvanffi (1913) makes a similar statement to the effect that conidia placed in water in the morning and maintained in obscure illumination germinate during the afternoon while those which were placed in strong light did not germinate until the following morning. Melhus (1911) states, however, that if the temperature is low, light will have no effect on germination.

In this connection the writer has conducted experiments and made certain observations. Conidia were placed on ice in a glass chamber and

exposed to strong sunlight but so far as could be observed the germination was as rapid as in the case of spores placed in an ice-box. Furthermore, conidia have been placed in a drop of water suspended from the cover of a Van Tiegham cell and the culture placed on the stage of the microscope, for continuous observation. In this case a strong light continually passes through the culture. By this method of procedure germination has been obtained in one-half to one hour. In our opinion the failure to germinate, apparently caused by strong light, may be due rather to a higher temperature. Indeed, Farlow (1876) states that the direct rays of the sun heats the water causing such rapid evaporation that germination could not occur.

According to Istvanffi (1913) a certain proportion of the conidia which do not germinate are still immature even though they may be of full-size. He says . . . (free translation) "the conidia in which karykinesis is already completed are not necessarily mature even though they have attained their full size. We have observed that the condia still undergo certain transformations if they are maintained in a moist atmosphere, namely, that the plasma becomes more reticulated in structure. These changes occupy about three or four hours. During this period the vacuoles become larger . . . and if examined in water the plasma appears granular."

It is certain that one who has studied conidial germination for some time will be able to state readily and with a fair degree of certainty, which conidia will not germinate. To describe this distinction is difficult but in general it may be said to be that the protoplasm of immature conidia appears more finely granular, denser and to have a much deeper greenish color. Before germination the protoplasm becomes more hyalin and less dense probably due to the appearance of the numerous vacuoles, noted by Istvanffi.

On the other hand the conidia may be too old to germinate, as has been pointed out previously by the writer (1913). Such conidia are characterized by the coarsely granular, brown protoplasm.

A third type of conidia, to which Istvanffi takes exception, has also been described by the writer, as "partly or wholly filled with a highly refractive drop which appears to be oleaginous in nature". Istvanffi admits that a conidium treated with osmic acid becomes a deep brown indicating the possible presence of some oily substance but he claims that the oil is present as minute drops. In this same connection both Viala (1893) and Patrigeon (1887) state that there are numerous brilliant or refringent drops present in the conidium. At the same time, Istvanffi states that the supposed error may be due to the fact that vacuoles are very abundant in the conidium and that these, being clear, "may resemble the ordinary refringence of the drops of oil." It will be noted that the writer stated that these refringent bodies remain in the conidium after germination. If vacuoles had been mistaken for independent bodies in the protoplasm, would they remain as such in an empty conidium? There is no question that in some cases, a clear, highly refringent, and well-defined body or "drop" remains in the conidium after germination. Its exact composition has not been determined but the possibilities are either oil or glycogen.

The vitality of the conidia varies with the conditions of humidity and temperature. Istvanffi states that at a temperature of 8° to 10° C. they will retain their vitality for two or three weeks and if placed directly upon

ice may be germinated after sixty days if optimum conditions be afforded. In warm, dry air they perish in five days.

The writer has germinated conidia which have been kept for two weeks at a temperature of 10° C., while those kept in a moist chamber at room temperature lost their vitality in from seven to ten days. It seems safe to assume that the conidia will retain their vitality in nature about a week.

The season makes no apparent difference in the germinative power of the conidia. As good germination has been obtained in October from conidia collected on wild vines as during the summer.

The germination of the conidia has been described by the writer (1913) but will be rewritten at this time in order that changes in certain details may be more intelligibly indicated. "At first the protoplasm is finely granular but about an hour after being placed in water there appear lighter hyaline spots in the protoplasm, which at the same time becomes a little denser and more granular." When stained with methylene blue it will readily be seen that the "hyaline spots" are the nuclei. "This continues until there is a dense granular mass with clear, distinct spots arranged at regular intervals. In a short time there appear in the protoplasm dark lines which mark out portions about each nucleus. These lines become more and more distinct and finally there are slight indentations along the margin of the previously smooth protoplasm. The content of the conidium is now very rough and irregular. By focusing, the individual swarmspores can be made out. In a few minutes the spores break apart and become distinct bodies. At this point there is a pause during which it seems that the spores must burst forth immediately and, coincidently, there seems to be a slight movement among them. Suddenly through the tip of the conidium there appears a bit of protoplasm of one of the swarmspores which slowly forces its way out until the opening becomes maximum in size when the entire mass of spores, jerkily but rapidly, slips out. It is quite certain that the opening is at the papilla, and is probably brought about by the dissolution of the wall at this point and not by its breaking, since no remnant of the wall can be found after evacuation." Istvanffi (1913) claims that the papilla is a cap-like structure which is pushed off when the zoospores emerge. The writer watched this process very closely and has fixed and stained conidia in all stages of germination but has never seen anything which might possibly be interpreted in this way.

During the preliminary stages of germination the conidium swells slightly, as Farlow (1876) has already intimated, probably due to the imbibition of water. Ordinarily the increase in size is about 1μ in both length and breadth. After the evacuation of the zoospores the size decreases about 2μ, becoming much smaller than the original conidium. Ultimately the conidial wall becomes greatly wrinkled and shriveled.

"The swarmspores remain for an instant at the end of the conidium and then pull apart and swim away." In this connection Farlow states, "They passed out rather slowly, usually one at a time, and paused for a moment in front of the opening, where they remained as if not yet quite free from one another. In a short time each segment began to extricate itself from the common mass, moved more and more actively, and finally darted off with great rapidity a full-fledged zoospore, furnished with two cilia." The writer states further, "It is quite probable that the flagella are formed

at this time by the pulling apart of the spores. There is a considerable jerking and wrenching before they separate. At times two spores remain attached for a long time and finally, by dint of much pulling, they snap apart and swim away. At other times as many as four or five spores are apparently joined together by their flagella. Thus it would seem that the flagella are slender threads of protoplasm pulled from the spores as they split apart." Clinton (1906) has made a similar suggestion in the case of Phytophthora phaseoli, videlicet, "The motion is due to a slender thread or a cilium drawn out by the pulling apart of the narrow zone connecting two adjacent bodies . . ." Istvanffi (1913) claims that the flagella are present on the spores before they emerge from the conidium and infers that there is no hesitation after emergence. He does not, however, figure them in this way. We have never seen the flagella on the newly formed zoospores in the conidium before evacuation though they were very clearly stained on those which were free and upon mature zoospores which were unable to escape from the conidium (vide post). Hence it is the writer's opinion that the flagella are produced during the period of emergence or while the zoospores are hesitating at the apex. The method of staining with methylene blue and carbol-fuchsin, has also revealed the fact that the spores are not connected by their flagella but by other minute strands of protoplasm (Pl. III, fig. 7).

"Sometimes all of the swarmspores do not escape from the conidium at once, but one or two remain swimming about within. These spores experience considerable difficulty, so to speak, in escaping, seeming to be unable to squeeze their nuclei through the opening. If they do manage to escape they are usually constricted at the center into a dumb-bell shape.

"The number of zoospores produced varies greatly. The large conidia may produce as many as fifteen to seventeen while some small ones have only one or two. The normal number is five to eight."

"The conidia containing the oil drop germinate readily at times. In this case the oil drop is left behind in the conidium, the entire mass of protoplasm going to make up the swarmspores."

"The shape of the swarmspores is plano-convex with a keel or ridge along the flat side. In the center near the flat surface there is a light hyaline spot, the nucleus. Near it, and less brilliant, is another spot which by tinting with stains appears to be a vacuole. From two points on each side of the nucleus arise the flagella, which are of unequal length (Pl. III, fig. 6)." Certain of the European investigators, Viala (1903) and more recently, Ravaz and Verge (1913), have stated that the flagella are terminated with a small lobe. According to the writer's observations this lobe is never present but at times the end of the flagellum may form a small loop which gives such an impression. In this connection, Istvanffi says ". . . Treated according to the methods of Zettnow, one may see that the ends of the cilia are not lobed as they are most often represented, their length is about 15 to 20μ."

"This is the normal shape of the spores but there are many exceptional shapes produced at times. It is quite apparent that when the spores are newly formed and escaping from the conidium, they are very plastic and capable of taking any form, but once they are fixed in any given shape it is difficult for them to change. Thus, if in pulling apart the spore becomes pyriform or some irregular shape it remains so."

"It has been stated by some authors that the swarmspores are constantly changing their shape when swimming about. It appears to the writer, however, that there is no actual change, the apparent change being due to the rolling motion of the spores as they swim. This, combined with their peculiar shape, produces the illusion."

"The size of the swarmspores varies, being 6 to 7 by 7.5 to 9μ. The flagella are from 27 to 33μ in length."

"After swimming about for approximately one-half hour the swarmspores gradually come to rest, round up and surround themselves with a thin membrane." It has been previously pointed out that they may continue active for a much longer time if the temperature is low. "They apparently do not immediately 'drop' their flagella but, rather, absorb them, because, after becoming globose, they continue to whirl and move back and forth until they gradually come to rest. They would probably stop abruptly if the flagella were immediately dropped. Staining which has clearly revealed the flagella on the motile forms, has failed to reveal them lying free among the quiescent forms in the same preparation."

"The spores remain globose for fifteen minutes, more or less, and then there appears a slight protuberance from one side. This is the germ-tube. It elongates and the contents of the spore follow. This continues until the contents have passed out into the tube, leaving the thin wall. After the tube reaches a certain length there may be produced an appressorium from which another tube arises later." In certain cases it has been observed that the swarmspores do not germinate but become very much more granular and finally seem to disintegrate entirely. At times this occurs almost to the exclusion of the germination.

"The germ-tube grows parallel to the surface of the leaf until a stomate is encountered when it turns sharp'y downward, passing through the opening into the leaf but never penetrates the epidermis directly. In order to pass through the stomatal opening the germ-tube becomes very greatly attenuated, being .4 to .8μ in diameter but swells immediately after passing into the sub-stomatal cavity forming a structure which, at times, closely resembles the germinating spore (Pl. I, figs. 1 to 11).

Pole-Evans (1907) has termed a similar structure in the case of the rusts, a sub-stomatal vesicle. Istvanffi interprets it in this case, however, as a secondary spore. Its assumed function may best be stated in his own words. "The physiological role of the secondary spore is in our estimation to assure the ulterior development of the germinating zoospores, for this reason all the protoplasm of the zoospore passes into the secondary spore which is well protected against all unfavorable exterior factors in the moist sub-stomatal cavity." This explanation of the peculiar behavior of the germ-tube seems very reasonable. The term "secondary spore" is, in the writer's opinion, also very apt because in many instances, in fact in practically all instances, it closely resembles the spore from which it arose. It germinates by means of one or more slender hyphae (Pl. I, figs. 2, 4, and 11), and according to the writer's observations does not produce haustoria hence does not serve to absorb food except that which may possibly be present in the intercellular spaces.

There are many variations in the size and form of the secondary spores. In some cases, especially in the leaves of susceptible varieties, the entire

cavity is filled by the irregular mass (Pl. I, fig. 5). In other cases the diameter is hardly more than that of the primary spore (Pl. I, fig. 1). In this case the shape is usually pyriform. The size varies from 6 to 22 by 17 to 27μ.

As has been indicated above there arise from the secondary spore one or more strands of mycelium which radiate in different directions into the tissue of the leaf. These do not average more than 2 to 3μ in diameter. The exact time elapsing before the haustoria are produced has not been determined but in all material examined by the writer they are not formed on the secondary spore as is figured by Istvanffi, nor are they developed from the mycelium immediately.

The incubation period varies with the weather, the variety, the season of the year and the portion of the plant affected. Istvanffi states that during the spring the incubation period is longest and that it gradually grows shorter until the early part of August after which it again lengthens. The periods as he determined them are May, 15 to 18 days, June, 11 to 14 days, July, 6 to 7 days, August, 5 to 6 days and late August to September, 9 to 11 days. On the fruit the period averages from 2 to 5 days longer.

The varieties Delaware, Niagara, Catawba and Clinton were inoculated during the summer of 1913. It was found that on the first three, which are relatively more susceptible than the last, that the period of incubation was from 7 to 12 days, while on the Clinton under the same conditions, the period was 20 days.

The difference exhibited in the incubation period on the Delaware and Clinton begins to be evident in the earliest stages of infection. Penetration of the germ-tube through the stomatal opening occurs in 3 to 5 hours in the leaves of the Delaware, while on the variety Clinton penetration apparently does not take place until after about 20 hours. In about 36 hours there is a considerable formation of mycelium in the former variety while in the latter the secondary spore has barely germinated (Pl. I, figs. 4 and 11). If this retarding of growth continues it easily explains the great difference in the incubation periods.

Following the method previously described by the writer (1913), numerous infection experiments were performed during the summer of 1913. Spore-laden drops of water were placed at all points on the upper and lower surfaces of the leaves. Notwithstanding the fact that the conditions under which the inoculations were made, were as nearly identical as it was possible to secure, in some cases the inoculations were on the upper and lower surfaces of different leaves in the same inoculation chamber, it was never possible to produce an infection through the upper surface of the leaf. The European investigators report a certain percentage of successful infections on this surface. Istvanffi has shown that this is true because stomates occur along the mid-rib and the principal veins and about the tips of the leaves, while on the lower surface they are very thickly and uniformly scattered over the surface. According to our observations on several different varieties there are no stomates on the upper surface of the leaves of American varieties. This would explain why it was not possible to infect the upper surface of the leaves.

Istvanffi has shown that there are a few stomates on the ovary of the flower of vinifera varieties and that they do not increase in number. When

the grape attains the size of a pea there are no stomates on its surface but in their place occur a number of lenticle-like structures. Thus we find again the reason that it is not possible to infect the berries except when they are very small. It can be shown without difficulty that the older berries become infected by mycelium which grows down through the pedicles and peduncles into the fruit. This view is further justified by the fact that there are a few stomates on the pedicle and peduncle thus allowing the possibility of infection at these points.

According to Müller-Thurgau (1911) slight tears or injuries may allow infection through the upper surface. He bases this statement on certain experiments in which this surface was punctured with a needle at the points of inoculation. To verify this statement similar experiments were conducted. Spore-laden drops of water were placed on the upper surface of the leaf at numerous points. In some cases the leaf was punctured with a needle through the drop and in other cases injured before the drop was placed upon the leaf. Checks of drops on the uninjured upper surface and on the lower surface were also run. No infections were obtained except through the lower surface. It was then thought that possibly the openings were not large enough. With this in view another series of experiments was conducted in which the leaf was torn as much as possible without allowing the water to penetrate to the lower surface. In some cases jagged tears fully 5 mm. long were made. As before the results were all negative. Hence in our opinion injuries to the upper surface do not permit infection unless the water is permitted to gain access to the lower surface through the opening so that the swarmspores may swim through the opening.

To determine whether the swarmspores produced from conidia which had fallen on the upper surface of the leaf, might be able to produce infection if conditions were such that they could gain access to the lower surface the following experiments were performed. The leaves were held with the lower surface firmly but not tightly against plates of perfectly clean glass. Drops of water were than so placed that there was a continuous layer passing from the upper surface over the margin and between the leaf and the glass. Fresh conidia were then placed in the water on the upper surface as far as possible from the margin. In three cases out of five (60 per cent) infections occurred. In the other cases the explanation of the failure lies in the fact that the film of water was broken on the upper surface of the leaf probably before conidial germination. This indicates that this method of infection is possible but that it may occur only in the most favorable conditions because of the ease with which the film is broken. This is an exact verification of the work of Ravaz and Verge (1911) relative to this same question.

Pathological Histology.

The chlorophyll, the plastids and at times the nucleus of the cell are eventually disintegrated. This process is gradual, being not only confined to certain cells or groups of cells, but even to the individual plastids within a cell, certain of them remaining green while others are entirely yellow. The first microscopical evidence of this change occurs in about three days after inoculation.

The cells of the spongy parenchyma are first to exhibit the indications of the disease, followed shortly by the palisade cells. The vascular tissue is never affected but the mesophyll cells immediately adjacent are first to be injured. This is particularly noticeable during dry weather. The epidermal cells may finally die, due rather to the death of the contiguous cells than to the direct effects of the mycelium.

Coincident with the loss of color the cell contents become plasmolysed, the wall collapses and finally the entire cell becomes brown. This effect seems to occur first in the palisade cells bordering the veins rather than in the spongy parenchyma, as one would expect, thus producing the peculiar network of brown lines so prominent on the upper surface within the yellow area. Gradually all the cells throughout the older portion of the spot are killed, causing the dead brown center. The collapsing of the diseased cells reduces the thickness of the leaf within the lesion to approximately one-half to two-thirds that of the normal.

The cortex, collenchyma, epidermis, medullary rays and pith of the stems are affected. Istvanffi (1913) states that the phloem and rarely the xylem may also be involved. The writer has never seen any evidence of the disease in these tissues. Farlow (1876) also states that all parts of the stem are affected except the wood. In the writer's opinion this difference can only be interpreted as further proof of the greater susceptibility of the European varieties.

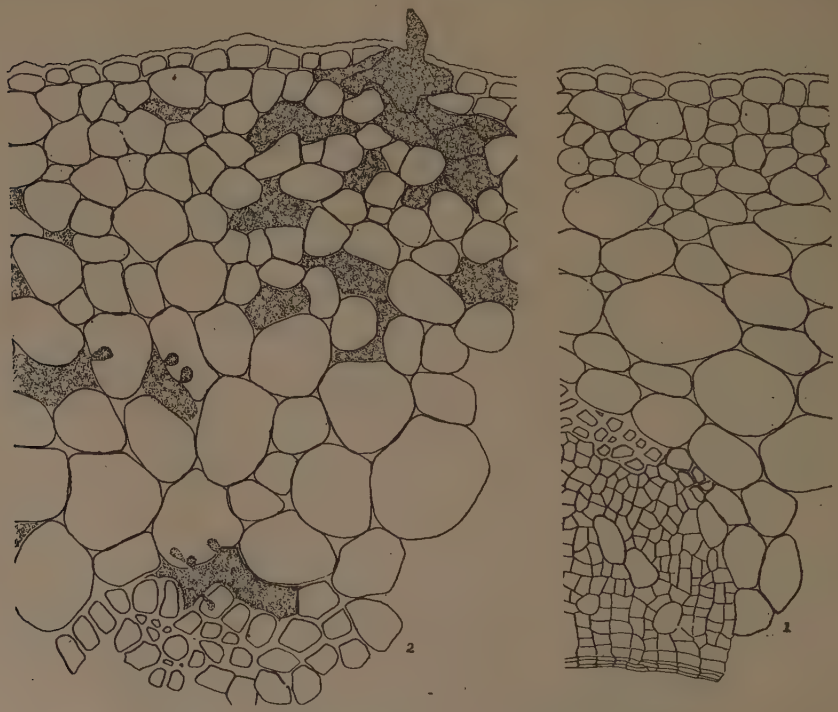

Plate V. Sections of healthy and diseased shoots.

The increase in the size of the stem is apparently not due to hyperplasia, but rather to a slight hypertrophy of the cells, and to the intussusception of a large amount of mycelium between the cells. (Pl. V, figs. 1 and 2). The water-soaked appearance of the diseased stems is in all probability the result of an excessive amount of sap in the intercellular spaces. This phenomenon may be caused by a change in permeability of the plasma membrane, possibly induced by the presence of the fungus.

As in the leaf the cells are ultimately killed and collapse, so producing the brown sunken areas in the stem.

In the livid berry the chlorophyll is acted upon as in the leaf and the green color lost, thus giving the peculiar blanched appearance so characteristic of this stage. In a short time the cells begin to collapse, the contents become more or less plasmolysed and the walls brown, resulting in the well-known soft, brown-rot stage.

The color of the prematurely ripened grapes is located in the outer six or seven layers of cells. It is due to the formation of some red substance in the cell-sap. This may occur in the cells immediately adjacent to the mycelium or several cells distant. It is very similar in appearance to the changes occurring in the normally ripening grape.

Pathological Physiology.

The question of the resistance or immunity of certain varieties of grapes, of different organs of the same variety, and of the same organ at different ages and seasons has been seriously considered and investigated for some time, particularly during the past five or six years.

Bottini (1909) determined that when leaves of susceptible varieties were immersed in or sprayed with the sap expressed from the leaves of a resistant variety, the former were less easily infected than when water was used. It is his opinion that the concentration of the sap of the resistant variety is the controlling factor in this case.

Laurent (1911) obtained the concentration of the cell sap of different parts of a vine and from different varieties. The method employed was to determine the freezing-point of the expressed juice, the freezing temperature, according to Livingston (1903), varying inversely with the concentration or the osmotic pressure. In this manner it was determined that the cell sap of the older leaves is much more concentrated than that of the younger leaves and that the sap of the berries is much less concentrated than either. In every case the least resistant parts of varieties contained less concentrated sap. With this as a basis he formulates the theory that resistance is the result of a more highly concentrated cell sap. He points out further that Müntz has stated that resistance becomes greater as the water content falls below 60 per cent.

Cercelet (1912), Capus (1913), Larue (1912) and many other European investigators are of the same opinion concerning the explanation of resistance. Cercelet states that nitrogen, inducing a succulent growth raises the water content and thus tends to render a vine more susceptible. On the other hand, potash and phosphate are said to increase the concentration of the cell-sap. Capus has made many interesting observations in this connection. He claims that vines grown on a gravelly soil are in general more resistant than those on a clay soil. In his opinion there are three stages of resistance in the

leaves, being susceptible when young, gaining immunity with maturity, and again becoming susceptible in the latter part of the season. The writer has also made a similar observation. This change is most strikingly exhibited in the more susceptible varieties. It does not occur, however, in young vines in a nursery nor is it so apparent in cold rainy seasons. He also claims that, unless the vine is in a receptive state, infection will not occur regardless of how favorable the other conditions may be. It is not clear, however, exactly what is meant by the condition of receptivity.

Istvanffi and Palinkas (1913) are of the opinion that susceptibility depends on the vapor tension in the sub-stomatal cavity and the other intercellular spaces, upon the turgor of the cells and to a certain extent upon the chemical composition of the cell sap, since chlorotic leaves are relatively resistant.

On the other hand, Averna-Sacca (1910) is of the opinion that the composition of the cell sap may be the important factor. To be more specific, he claims that the acidity plays an important part in rendering a vine resistant. In support of this contention he points out that the acidity of resistant forms varied from 4.3 to 10.3 per cent, whereas the acidity of the more susceptible varieties is only .5 to 2.6 per cent.

Resistance to the mildew does not seem to be due to any morphological characters of the leaf. The densely matted hairs on the lower surface of the leaf of certain varieties cannot be a factor, since it has been determined that small drops of water readily permeate them to the surface of the leaf. Furthermore many of our most resistant forms are smooth leaved while certain of those which are most susceptible are densely clothed with hairs. The writer has determined that there are several thousand more stomates per square centimeter on the leaves of certain susceptible varieties than on those of resistant forms but in any case they are amply sufficient to permit ready infection.

Infection experiments may offer some explanation of resistance. In the case of the Delawares the germ tube very readily penetrates the stomate and makes a rapid and prolific growth within the leaf, whereas after the tube has penetrated the stomate of the leaf on a Clinton it seems to encounter some influence which checks its growth, the substomatal swelling is smaller, the secondary mycelium is more attenuated and the growth during a given period is much less.

A consideration of the difference in the abundance of fruiting on the leaves of susceptible and resistant varieties is also significant. It is evident that in order to produce an abundance of spores the fungus must absorb sufficient food material and an insufficient or unavailable supply will reduce the number of spores. Granting this we may say that the food in the resistant forms is not available or that some influence is present which resists the assimilation of the available food, because the fructification on the resistant varieties is strikingly less than on the more susceptible forms.

The apparent immunity of the berries is due to the waxy covering which hinders the adhesion of water to their surface and to the lack of stomates. That this resistance is only apparent immediately becomes evident if the fungus obtains entrance in some other way. The growth of the mycelium is very rapid and the berry is quickly rotted.

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Plate I.

Infection of the grape leaf by Plasmopara viticola.

Figs. 1, 2, 4 and 5 are on the leaves of the variety Delaware; the others are on the variety Clinton. x 750.

Fig. 1. Seven hours after inoculation at 50° F.

Fig. 2. Twenty hours after inoculation at 50° F.

Fig. 3. Seven hours after inoculation at 50° F.

Fig. 4. Thirty-six hours after inoculation at 70° F.

Fig. 5. Thirty-six hours after inoculation at 70° F.

Fig. 6. Thirty-six hours after inoculation at 50° F.

Fig. 7. Twenty hours after inoculation at 50° F.

Fig. 8. Twenty hours after inoculation at 50° F.

Fig. 9. Thirty-four hours after inoculation at 50° F.

Fig. 10. Thirty hours after inoculation at 50° F.

Fig. 11. Thirty-six hours after inoculation at 50° F.

Plate II.

Haustoria and mycelium of Plasmopara viticola.

Fig. 1. A haustorium which is still enclosed in the sheath of host cell-wall. x 650.

Figs. 2-5. Showing the goblet-shaped sheath about the base of the haustoria. In figures 2, 4 and 5 the plasma membrane is pressed inward but not penetrated. x 750.

Fig. 6. The formation of the mycelial cushion beneath the stomate, previous to conidiophore formation. x 750.

Fig. 7. The mycelium bursting through the epidermis of the petiole to form conidiophores. x 750.

Plate III.

Fructifications of Plasmopara viticola.

Figs. 1 and 3 show the effects of cuprammonia on the basal portion of the conidiophore. x 300.

Fig. 2. The basal portion of a normal conidiophore. x 300.

Fig. 4. Showing the comparatively slight effect of cuprammonia on the distal end of the conidiophore. x 300.

Fig. 5. A conidium attached to the sterigma showing the lenticular disjunctive region. x 750.

Figs. 6 and 7. The zoospores. In figure 7 two of the zoospores are attached by a slender strand of protoplasm. Figure 6 x 750. Figure 7 x 375.

Figs. 8-10. The germination of the conidia by zoospores.

Fig. 11. Conidia produced by the germination of the oospore.

Plate IV.

A few stages in the development of the oospore.

Fig. 1. A multinucleate oogonium. The nuclei are apparently zonate. Probably following a division since there are disintegrating nuclei occurring abundantly. x 750.

Fig. 2. An oogonium and antheridium. x 300.

Fig. 3. An oospore containing a single nucleus lying near a dark body which may be interpreted as the coenocentrum. Lying outside of the thick wall are remains of extraneous nuclei. x 750.

Fig. 4. A single nucleated oospore which contains a coenocentrum and what appear to be remnants of other nuclei lying near. About the margin are other dark bodies, probably the remains of nuclei which migrated from the centrosphere. x 750.

Fig. 5. An immature oospore about which the endosporium is being formed. There is an abundant periplasm present. x 750.

Fig. 6. A practically mature oospore. x 750.

Plate V.

Sections of healthy and diseased shoots.

Fig. 1. A cross-section of a healthy petiole of a leaf. x 300.

Fig. 2. A cross-section of the diseased petiole of approximately the same age as that shown in figure 1. This section also was taken from the same position on the petiole. The intussusception of the mycelium very largely explains the hypertrophy of the diseased petiole. x 300.